

V. Details of One Embodiment of
A Fluorescent Detection Device

Fig. 9 illustrates a fluorescent detection device for detecting fluorescently labeled receptors on a substrate. A substrate 112 is placed on an x/y translation table 202. In a preferred embodiment the x/y translation table is a model no. PM500-A1 manufactured by Newport Corporation. The x/y translation table is connected to and controlled by an appropriately programmed digital computer 204 which may be, for example, an appropriately programmed IBM PC/AT or AT compatible computer. Of course, other computer systems, special purpose hardware, or the like could readily be substituted for the AT computer used herein for illustration. Computer software for the translation and data collection functions described herein can be provided based on commercially available software including, for example, "Lab Windows" licensed by National Instruments, which is incorporated herein by reference for all purposes.

The substrate and x/y translation table are placed under a microscope 206 which includes one or more objectives 208. Light (about 488 nm) from a laser 210, which in some embodiments is a model no. 2020-05 argon ion laser manufactured by Spectraphysics, is directed at the substrate by a dichroic mirror 207 which passes greater than about 520 nm light but reflects 488 nm light. Dichroic mirror 207 may be, for example, a model no. FT510 manufactured by Carl Zeiss. Light reflected from the mirror then enters the microscope 206 which may be, for example, a model no. Axioscop 20 manufactured by Carl Zeiss. Fluorescein-marked materials on the substrate will fluoresce >488 nm light, and the fluoresced light will be collected by the microscope and passed through the mirror. The fluorescent light from the substrate is then directed through a wavelength filter 209 and, thereafter through an aperture plate 211.

Wavelength filter 209 may be, for example, a model no. OG530 manufactured by Melles Griot and aperture plate 211 may be, for example, a model no. 477352/477380 manufactured by Carl Zeiss.

5 The fluoresced light then enters a
photomultiplier tube 212 which in some embodiments is a
model no. R943-02 manufactured by Hamamatsu, the signal
is amplified in preamplifier 214 and photons are counted
by photon counter 216. The number of photons is recorded
10 as a function of the location in the computer 204.
Pre-Amp 214 may be, for example, a model no. SR440
manufactured by Stanford Research Systems and photon
counter 216 may be a model no. SR400 manufactured by
Stanford Research Systems. The substrate is then moved
15 to a subsequent location and the process is repeated.
In preferred embodiments the data are acquired every 1 to
100 μm with a data collection diameter of about 0.8 to
10 μm preferred. In embodiments with sufficiently high
fluorescence, a CCD detector with broadfield illumination
20 is utilized.

By counting the number of photons generated in
a given area in response to the laser, it is possible to
determine where fluorescent marked molecules are located
on the substrate. Consequently, for a slide which has a
25 matrix of polypeptides, for example, synthesized on the
surface thereof, it is possible to determine which of the
polypeptides is complementary to a fluorescently marked
receptor.

According to preferred embodiments, the
30 intensity and duration of the light applied to the
substrate is controlled by varying the laser power and
scan stage rate for improved signal-to-noise ratio by
maximizing fluorescence emission and minimizing
background noise.

35 While the detection apparatus has been
illustrated primarily herein with regard to the detection
of marked receptors, the invention will find application

in other areas. For example, the detection apparatus disclosed herein could be used in the fields of catalysis, DNA or protein gel scanning, and the like.

5 VI. Determination of Relative
Binding Strength of Receptors

The signal-to-noise ratio of the present invention is sufficiently high that not only can the presence or absence of a receptor on a ligand be
10 detected, but also the relative binding affinity of receptors to a variety of sequences can be determined.

In practice it is found that a receptor will bind to several peptide sequences in an array, but will bind much more strongly to some sequences than others.
15 Strong binding affinity will be evidenced herein by a strong fluorescent or radiographic signal since many receptor molecules will bind in a region of a strongly bound ligand. Conversely, a weak binding affinity will be evidenced by a weak fluorescent or radiographic signal
20 due to the relatively small number of receptor molecules which bind in a particular region of a substrate having a ligand with a weak binding affinity for the receptor. Consequently, it becomes possible to determine relative binding avidity (or affinity in the case of univalent
25 interactions) of a ligand herein by way of the intensity of a fluorescent or radiographic signal in a region containing that ligand.

Semiquantitative data on affinities might also be obtained by varying washing conditions and
30 concentrations of the receptor. This would be done by comparison to known ligand receptor pairs, for example.

VII. Examples

The following examples are provided to
35 illustrate the efficacy of the inventions herein. All operations were conducted at about ambient temperatures and pressures unless indicated to the contrary.

A. Slide Preparation

Before attachment of reactive groups it is preferred to clean the substrate which is, in a preferred embodiment a glass substrate such as a microscope slide or cover slip. According to one embodiment the slide is soaked in an alkaline bath consisting of, for example, 1 liter of 95% ethanol with 120 ml of water and 120 grams of sodium hydroxide for 12 hours. The slides are then washed under running water and allowed to air dry, and rinsed once with a solution of 95% ethanol.

The slides are then aminated with, for example, aminopropyltriethoxysilane for the purpose of attaching amino groups to the glass surface on linker molecules, although any omega functionalized silane could also be used for this purpose. In one embodiment 0.1% aminopropyltriethoxysilane is utilized, although solutions with concentrations from $10^{-7}\%$ to 10% may be used, with about $10^{-3}\%$ to 2% preferred. A 0.1% mixture is prepared by adding to 100 ml of a 95% ethanol/5% water mixture, 100 microliters (μ l) of aminopropyltriethoxysilane. The mixture is agitated at about ambient temperature on a rotary shaker for about 5 minutes. 500 μ l of this mixture is then applied to the surface of one side of each cleaned slide. After 4 minutes, the slides are decanted of this solution and rinsed three times by dipping in, for example, 100% ethanol.

After the plates dry, they are placed in a 110-120°C vacuum oven for about 20 minutes, and then allowed to cure at room temperature for about 12 hours in an argon environment. The slides are then dipped into DMF (dimethylformamide) solution, followed by a thorough washing with methylene chloride.

The aminated surface of the slide is then exposed to about 500 μ l of, for example, a 30 millimolar (mM) solution of NVOC-GABA (gamma amino butyric acid) NHS (N-hydroxysuccinimide) in DMF for attachment of a NVOC-GABA to each of the amino groups.

The surface is washed with, for example, DMF, methylene chloride, and ethanol.

Any unreacted aminopropyl silane on the surface--that is, those amino groups which have not had the NVOC-GABA attached--are now capped with acetyl groups (to prevent further reaction) by exposure to a 1:3 mixture of acetic anhydride in pyridine for 1 hour. Other materials which may perform this residual capping function include trifluoroacetic anhydride, formicacetic anhydride, or other reactive acylating agents. Finally, the slides are washed again with DMF, methylene chloride, and ethanol.

B. Synthesis of Eight Trimers of "A" and "B"

Fig. 10 illustrates a possible synthesis of the eight trimers of the two-monomer set: gly, phe (represented by "A" and "B," respectively). A glass slide bearing silane groups terminating in 6-nitro-veratryloxycarboxamide (NVOC-NH) residues is prepared as a substrate. Active esters (pentafluorophenyl, OBt, etc.) of gly and phe protected at the amino group with NVOC are prepared as reagents. While not pertinent to this example, if side chain protecting groups are required for the monomer set, these must not be photoreactive at the wavelength of light used to protect the primary chain.

For a monomer set of size n , $n \times \ell$ cycles are required to synthesize all possible sequences of length ℓ . A cycle consists of:

1. Irradiation through an appropriate mask to expose the amino groups at the sites where the next residue is to be added, with appropriate washes to remove the by-products of the deprotection.
2. Addition of a single activated and protected (with the same photochemically-removable group) monomer, which will react

only at the sites addressed in step 1, with appropriate washes to remove the excess reagent from the surface.

The above cycle is repeated for each member of the monomer set until each location on the surface has been extended by one residue in one embodiment. In other embodiments, several residues are sequentially added at one location before moving on to the next location. Cycle times will generally be limited by the coupling reaction rate, now as short as 20 min in automated peptide synthesizers. This step is optionally followed by addition of a protecting group to stabilize the array for later testing. For some types of polymers (e.g., peptides), a final deprotection of the entire surface (removal of photoprotective side chain groups) may be required.

More particularly, as shown in Fig. 10A, the glass 20 is provided with regions 22, 24, 26, 28, 30, 32, 34, and 36. Regions 30, 32, 34, and 36 are masked, as shown in Fig. 10B and the glass is irradiated and exposed to a reagent containing "A" (e.g., gly), with the resulting structure shown in Fig. 10C. Thereafter, regions 22, 24, 26, and 28 are masked, the glass is irradiated (as shown in Fig. 10D) and exposed to a reagent containing "B" (e.g., phe), with the resulting structure shown in Fig. 10E. The process proceeds, consecutively masking and exposing the sections as shown until the structure shown in Fig. 10M is obtained. The glass is irradiated and the terminal groups are, optionally, capped by acetylation. As shown, all possible trimers of gly/phe are obtained.

In this example, no side chain protective group removal is necessary. If it is desired, side chain deprotection may be accomplished by treatment with ethanedithiol and trifluoroacetic acid.

In general, the number of steps needed to obtain a particular polymer chain is defined by:

$$n \times \ell \quad (1)$$

where:

5 n = the number of monomers in the basis set of monomers, and

ℓ = the number of monomer units in a polymer chain.

 Conversely, the synthesized number of sequences of length ℓ will be:

$$n^{\ell}. \quad (2)$$

 Of course, greater diversity is obtained by using masking strategies which will also include the synthesis of polymers having a length of less than ℓ . If, in the extreme case, all polymers having a length less than or equal to ℓ are synthesized, the number of polymers synthesized will be:

$$n^{\ell} + n^{\ell-1} + \dots + n^1. \quad (3)$$

 The maximum number of lithographic steps needed will generally be n for each "layer" of monomers, i.e., the total number of masks (and, therefore, the number of lithographic steps) needed will be $n \times \ell$. The size of the transparent mask regions will vary in accordance with the area of the substrate available for synthesis and the number of sequences to be formed. In general, the size of the synthesis areas will be:

$$\text{size of synthesis areas} = (A)/(S)$$

where:

35 A is the total area available for synthesis;

and

S is the number of sequences desired in the area.

It will be appreciated by those of skill in the art that the above method could readily be used to simultaneously produce thousands or millions of oligomers on a substrate using the photolithographic techniques disclosed herein. Consequently, the method results in the ability to practically test large numbers of, for example, di, tri, tetra, penta, hexa, hepta, octapeptides, dodecapeptides, or larger polypeptides (or correspondingly, polynucleotides).

The above example has illustrated the method by way of a manual example. It will of course be appreciated that automated or semi-automated methods could be used. The substrate would be mounted in a flow cell for automated addition and removal of reagents, to minimize the volume of reagents needed, and to more carefully control reaction conditions. Successive masks could be applied manually or automatically.

C. Synthesis of a Dimer of an Aminopropyl Group and a Fluorescent Group

In synthesizing the dimer of an aminopropyl group and a fluorescent group, a functionalized durapore membrane was used as a substrate. The durapore membrane was a polyvinylidene difluoride with aminopropyl groups. The aminopropyl groups were protected with the DDZ group by reaction of the carbonyl chloride with the amino groups, a reaction readily known to those of skill in the art. The surface bearing these groups was placed in a solution of THF and contacted with a mask bearing a checkerboard pattern of 1 mm opaque and transparent regions. The mask was exposed to ultraviolet light having a wavelength down to at least about 280 nm for about 5 minutes at ambient temperature, although a wide range of exposure times and temperatures may be

appropriate in various embodiments of the invention. For example, in one embodiment, an exposure time of between about 1 and 5000 seconds may be used at process temperatures of between -70 and +50°C.

5 In one preferred embodiment, exposure times of between about 1 and 500 seconds at about ambient pressure are used. In some preferred embodiments, pressure above ambient is used to prevent evaporation.

10 The surface of the membrane was then washed for about 1 hour with a fluorescent label which included an active ester bound to a chelate of a lanthanide. Wash times will vary over a wide range of values from about a few minutes to a few hours. These materials fluoresce in the red and the green visible region. After the
15 reaction with the active ester in the fluorophore was complete, the locations in which the fluorophore was bound could be visualized by exposing them to ultraviolet light and observing the red and the green fluorescence. It was observed that the derivatized regions of the
20 substrate closely corresponded to the original pattern of the mask.

D. Demonstration of Signal Capability

25 Signal detection capability was demonstrated using a low-level standard fluorescent bead kit manufactured by Flow Cytometry Standarda and having model no. 824. This kit includes 5.8 μm diameter beads, each impregnated with a known number of fluorescein molecules.

30 One of the beads was placed in the illumination field on the scan stage as shown in Fig. 9 in a field of a laser spot which was initially shuttered. After being positioned in the illumination field, the photon detection equipment was turned on. The laser beam was unblocked and it interacted with the particle bead,
35 which then fluoresced. Fluorescence curves of beads impregnated with 7,000; 13,000; and 29,000 fluorescein molecules, are shown in Figs. 11A, 11B, and 11C

respectively. On each curve, traces for beads without fluorescein molecules are also shown. These experiments were performed with 488 nm excitation, with 100 μ W of laser power. The light was focused through a 40 power 0.75 NA objective.

The fluorescence intensity in all cases started off at a high value and then decreased exponentially. The fall-off in intensity is due to photobleaching of the fluorescein molecules. The traces of beads without fluorescein molecules are used for background subtraction. The difference in the initial exponential decay between labeled and nonlabeled beads is integrated to give the total number of photon counts, and this number is related to the number of molecules per bead. Therefore, it is possible to deduce the number of photons per fluorescein molecule that can be detected. For the curves illustrated in Fig. 11, this calculation indicates the radiation of about 40 to 50 photons per fluorescein molecule are detected.

E. Determination of the Number of Molecules Per Unit Area

Aminopropylated glass microscope slides prepared according to the methods discussed above were utilized in order to establish the density of labeling of the slides. The free amino termini of the slides were reacted with FITC (fluorescein isothiocyanate) which forms a covalent linkage with the amino group. The slide is then scanned to count the number of fluorescent photons generated in a region which, using the estimated 40-50 photons per fluorescent molecule, enables the calculation of the number of molecules which are on the surface per unit area.

A slide with aminopropyl silane on its surface was immersed in a 1 mM solution of FITC in DMF for 1 hour at about ambient temperature. After reaction, the slide was washed twice with DMF and then washed with

ethanol, water, and then ethanol again. It was then dried and stored in the dark until it was ready to be examined.

Through the use of curves similar to those shown in Fig. 11, and by integrating the fluorescent counts under the exponentially decaying signal, the number of free amino groups on the surface after derivitization was determined. It was determined that slides with labeling densities of 1 fluoroscein per $10^3 \times 10^3$ to $\sim 2 \times 2$ nm could be reproducibly made as the concentration of aminopropyltriethoxysilane varied from $10^{-5}\%$ to $10^{-1}\%$.

F. Removal of NVOC and Attachment of
A Fluorescent Marker

NVOC-GABA groups were attached as described above. The entire surface of one slide was exposed to light so as to expose a free amino group at the end of the gamma amino butyric acid. This slide, and a duplicate which was not exposed, were then exposed to fluorescein isothiocyanate (FITC).

Fig. 12A illustrates the slide which was not exposed to light, but which was exposed to FITC. The units of the x axis are time and the units of the y axis are counts. The trace contains a certain amount of background fluorescence. The duplicate slide was exposed to 350 nm broadband illumination for about 1 minute (12 mW/cm^2 , ~ 350 nm illumination), washed and reacted with FITC. The fluorescence curves for this slide are shown in Fig. 12B. A large increase in the level of fluorescence is observed, which indicates photolysis has exposed a number of amino groups on the surface of the slides for attachment of a fluorescent marker.

G. Use of a Mask in Removal of NVOC

The next experiment was performed with a 0.1% aminopropylated slide. Light from a Hg-Xe arc lamp was imaged onto the substrate through a laser-ablated chrome-on-glass mask in direct contact with the substrate.

This slide was illuminated for approximately 5 minutes, with 12 mW of 350 nm broadband light and then reacted with the 1 mM FITC solution. It was put on the laser detection scanning stage and a graph was plotted as a two-dimensional representation of position color-coded for fluorescence intensity. The fluorescence intensity (in counts) as a function of location is given on the color scale to the right of Figure 13A for a mask having 100x100 μm squares.

The experiment was repeated a number of times through various masks. The fluorescence pattern for a 50 μm mask is illustrated in Fig. 13B, for a 20 μm mask in Fig. 13C, and for a 10 μm mask in Fig. 13D. The mask pattern is distinct down to at least about 10 μm squares using this lithographic technique.

H. Attachment of YGGFL and Subsequent Exposure to Herz Antibody and Goat Antimouse

In order to establish that receptors to a particular polypeptide sequence would bind to a surface-bound peptide and be detected, Leu enkephalin was coupled to the surface and recognized by an antibody. A slide was derivatized with 0.1% amino propyl-triethoxysilane and protected with NVOC. A 500 μm checkerboard mask was used to expose the slide in a flow cell using backside contact printing. The Leu enkephalin sequence (H_2N -tyrosine, glycine, glycine, phenylalanine, leucine- CO_2H , otherwise referred to herein as YGGFL) was attached via its carboxy end to the exposed amino groups on the surface of the slide. The peptide was added in DMF solution with the BOP/HOBT/DIEA coupling reagents and

recirculated through the flow cell for 2 hours at room temperature.

A first antibody, known as the Herz antibody, was applied to the surface of the slide for 45 minutes at 2 μ g/ml in a supercocktail (containing 1% BSA and 1% ovalbumin also in this case). A second antibody, goat anti-mouse fluorescein conjugate, was then added at 2 μ g/ml in the supercocktail buffer, and allowed to incubate for 2 hours.

The results of this experiment are provided in Fig. 14. Again, this figure illustrates fluorescence intensity as a function of position. The fluorescence scale is shown on the right, according to the color coding. This image was taken at 10 μ m steps. This figure indicates that not only can deprotection be carried out in a well defined pattern, but also that (1) the method provides for successful coupling of peptides to the surface of the substrate, (2) the surface of a bound peptide is available for binding with an antibody, and (3) that the detection apparatus capabilities are sufficient to detect binding of a receptor.

I. Monomer-by-Monomer Formation of YGGFL and Subsequent Exposure to Labeled Antibody

Monomer-by-monomer synthesis of YGGFL and GGFL in alternate squares was performed on a slide in a checkerboard pattern and the resulting slide was exposed to the Herz antibody. This experiment and the results thereof are illustrated in Figs. 15A, 15B, 15C, and 15D.

In Fig. 15A, a slide is shown which is derivatized with the aminopropyl group, protected in this case with t-BOC (t-butoxycarbonyl). The slide was treated with TFA to remove the t-BOC protecting group. E-aminocaproic acid, which was t-BOC protected at its amino group, was then coupled onto the aminopropyl groups. The aminocaproic acid serves as a spacer between the aminopropyl group and the peptide to be synthesized.

The amino end of the spacer was deprotected and coupled to NVOC-leucine. The entire slide was then illuminated with 12 mW of 325 nm broadband illumination. The slide was then coupled with NVOC-phenylalanine and washed. The entire slide was again illuminated, then coupled to NVOC-glycine and washed. The slide was again illuminated and coupled to NVOC-glycine to form the sequence shown in the last portion of Fig. 15A.

As shown in Fig. 15B, alternating regions of the slide were then illuminated using a projection print using a 500x500 μm checkerboard mask; thus, the amino group of glycine was exposed only in the lighted areas. When the next coupling chemistry step was carried out, NVOC-tyrosine was added, and it coupled only at those spots which had received illumination. The entire slide was then illuminated to remove all the NVOC groups, leaving a checkerboard of YGGFL in the lighted areas and in the other areas, GGFL. The Herz antibody (which recognizes the YGGFL, but not GGFL) was then added, followed by goat anti-mouse fluorescein conjugate.

The resulting fluorescence scan is shown in Fig. 15C, and the color coding for the fluorescence intensity is again given on the right. Dark areas contain the tetrapeptide GGFL, which is not recognized by the Herz antibody (and thus there is no binding of the goat anti-mouse antibody with fluorescein conjugate), and in the red areas YGGFL is present. The YGGFL pentapeptide is recognized by the Herz antibody and, therefore, there is antibody in the lighted regions for the fluorescein-conjugated goat anti-mouse to recognize.

Similar patterns are shown for a 50 μm mask used in direct contact ("proximity print") with the substrate in Fig. 15D. Note that the pattern is more distinct and the corners of the checkerboard pattern are touching when the mask is placed in direct contact with the substrate (which reflects the increase in resolution using this technique).

J. Monomer-by-Monomer Synthesis of YGGFL and PGGFL

A synthesis using a 50 μm checkerboard mask similar to that shown in Fig. 15 was conducted. However, P was added to the GGFL sites on the substrate through an additional coupling step. P was added by exposing protected GGFL to light through a mask, and subsequent exposure to P in the manner set forth above. Therefore, half of the regions on the substrate contained YGGFL and the remaining half contained PGGFL.

The fluorescence plot for this experiment is provided in Fig. 16. As shown, the regions are again readily discernable. This experiment demonstrates that antibodies are able to recognize a specific sequence and that the recognition is not length-dependent.

K. Monomer-by-Monomer Synthesis of YGGFL and YPGGFL

In order to further demonstrate the operability of the invention, a 50 μm checkerboard pattern of alternating YGGFL and YPGGFL was synthesized on a substrate using techniques like those set forth above. The resulting fluorescence plot is provided in Fig. 17. Again, it is seen that the antibody is clearly able to recognize the YGGFL sequence and does not bind significantly at the YPGGFL regions.

L. Synthesis of an Array of Sixteen Different Amino Acid Sequences and Estimation of Relative Binding Affinity to Herz Antibody

Using techniques similar to those set forth above, an array of 16 different amino acid sequences (replicated four times) was synthesized on each of two glass substrates. The sequences were synthesized by attaching the sequence NVOC-GFL across the entire surface of the slides. Using a series of masks, two layers of amino acids were then selectively applied

to the substrate. Each region had dimensions of 0.25 cm x 0.0625 cm. The first slide contained amino acid sequences containing only L amino acids while the second slide contained selected D amino acids. Figs. 18A and 18B illustrate a map of the various regions on the first and second slides, respectively. The patterns shown in Figs. 18A and 18B were duplicated four times on each slide. The slides were then exposed to the Herz antibody and fluorescein-labeled goat anti-mouse.

Fig. 19 is a fluorescence plot of the first slide, which contained only L amino acids. Red indicates strong binding (149,000 counts or more) while black indicates little or no binding of the Herz antibody (20,000 counts or less). The bottom right-hand portion of the slide appears "cut off" because the slide was broken during processing. The sequence YGGFL is clearly most strongly recognized. The sequences YAGFL and YSGFL also exhibit strong recognition of the antibody. By contrast, most of the remaining sequences show little or no binding. The four duplicate portions of the slide are extremely consistent in the amount of binding shown therein.

Fig. 20 is a fluorescence plot of the second slide. Again, strongest binding is exhibited by the YGGFL sequence. Significant binding is also detected to YAGFL, YSGFL, and YpGFL. The remaining sequences show less binding with the antibody. Note the low binding efficiency of the sequence yGGFL.

Table 6 lists the various sequences tested in order of relative fluorescence, which provides information regarding relative binding affinity.

Table 6
Apparent Binding to Herz Ab

	<u>L-a.a. Set</u>	<u>D-a.a. Set</u>
5	YGGFL	YGGFL
	YAGFL	YaGFL
	YSGFL	YsGFL
	LGGFL	YpGFL
	FGGFL	fGGFL
10	YPGFL	yGGFL
	LAGFL	faGFL
	FAGFL	wGGFL
	WGGFL	yaGFL
		fpGFL
15		waGFL

VIII. Illustrative Alternative Embodiment

20 According to an alternative embodiment of the invention, the methods provide for attaching to the surface a caged binding member which in its caged form has a relatively low affinity for other potentially binding species, such as receptors and specific binding
25 substances. Such techniques are more fully described in copending application Serial No. 404,920, filed September 8, 1989, and incorporated herein by reference for all purposes.

30 According to this alternative embodiment, the invention provides methods for forming predefined regions on a surface of a solid support, wherein the predefined regions are capable of immobilizing receptors. The methods make use of caged binding members attached to the surface to enable selective activation of the predefined
35 regions. The caged binding members are liberated to act as binding members ultimately capable of binding receptors upon selective activation of the predefined

regions. The activated binding members are then used to immobilize specific molecules such as receptors on the predefined region of the surface. The above procedure is repeated at the same or different sites on the surface so as to provide a surface prepared with a plurality of regions on the surface containing, for example, the same or different receptors. When receptors immobilized in this way have a differential affinity for one or more ligands, screenings and assays for the ligands can be conducted in the regions of the surface containing the receptors.

The alternative embodiment may make use of novel caged binding members attached to the substrate. Caged (unactivated) members have a relatively low affinity for receptors of substances that specifically bind to uncaged binding members when compared with the corresponding affinities of activated binding members. Thus, the binding members are protected from reaction until a suitable source of energy is applied to the regions of the surface desired to be activated. Upon application of a suitable energy source, the caging groups labilize, thereby presenting the activated binding member. A typical energy source will be light.

Once the binding members on the surface are activated they may be attached to a receptor. The receptor chosen may be a monoclonal antibody, a nucleic acid sequence, a drug receptor, etc. The receptor will usually, though not always, be prepared so as to permit attaching it, directly or indirectly, to a binding member. For example, a specific binding substance having a strong binding affinity for the binding member and a strong affinity for the receptor or a conjugate of the receptor may be used to act as a bridge between binding members and receptors if desired. The method uses a receptor prepared such that the receptor retains its activity toward a particular ligand.

Preferably, the caged binding member attached to the solid substrate will be a photoactivatable biotin complex, i.e., a biotin molecule that has been chemically modified with photoactivatable protecting groups so that it has a significantly reduced binding affinity for avidin or avidin analogs than does natural biotin. In a preferred embodiment, the protecting groups localized in a predefined region of the surface will be removed upon application of a suitable source of radiation to give binding members, that are biotin or a functionally analogous compound having substantially the same binding affinity for avidin or avidin analogs as does biotin.

In another preferred embodiment, avidin or an avidin analog is incubated with activated binding members on the surface until the avidin binds strongly to the binding members. The avidin so immobilized on predefined regions of the surface can then be incubated with a desired receptor or conjugate of a desired receptor. The receptor will preferably be biotinylated, e.g., a biotinylated antibody, when avidin is immobilized on the predefined regions of the surface. Alternatively, a preferred embodiment will present an avidin/biotinylated receptor complex, which has been previously prepared, to activated binding members on the surface.

IX. Conclusion

The present inventions provide greatly improved methods and apparatus for synthesis of polymers on substrates. It is to be understood that the above description is intended to be illustrative and not restrictive. Many embodiments will be apparent to those of skill in the art upon reviewing the above description. By way of example, the invention has been described primarily with reference to the use of photoremovable protective groups, but it will be readily recognized by those of skill in the art that sources of radiation other than light could also be used. For example, in some

embodiments it may be desirable to use protective groups which are sensitive to electron beam irradiation, x-ray irradiation, in combination with electron beam lithograph, or x-ray lithography techniques.

5 Alternatively, the group could be removed by exposure to an electric current. The scope of the invention should, therefore, be determined not with reference to the above description, but should instead be determined with reference to the appended claims, along with the
10 full scope of equivalents to which such claims are entitled.

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WHAT IS CLAIMED IS:

1. A method of preparing sequences on a substrate comprising the steps of:

- 5 a) exposing a first region of said substrate to an activator to remove a protective group;
- b) exposing at least said first region to a first monomer;
- 10 c) exposing a second region to an activator to remove a protective group; and
- d) exposing at least said second region to a second monomer.

2. The method as recited in claim 1 wherein said
15 steps of exposing to an activator use an activator selected from the group consisting of ion beams, electron beams, gamma rays, x-rays, ultra-violet radiation, light, infra-red radiation, microwaves, electric currents, radiowaves, and combinations thereof.

20 3. The method as recited in claim 1 wherein said protective groups are photosensitive protective groups.

25 4. The method as recited in claim 1 wherein said steps of exposing to an activator are steps of applying light to selected regions of said substrate.

30 5. The method as recited in claim 1 wherein said first and the second monomers are amino acids.

35 6. The method as recited in claim 1 further comprising a step of screening sequences on said substrate for affinity with a receptor, said step of screening further comprising the step of exposing said substrate to said receptor and testing for the presence of said receptor in said first and said second region.

7. The method as recited in claim 6 wherein said receptor is an antibody.

8. The method as recited in claim 1 wherein said substrate is selected from the group consisting of polymerized Langmuir Blodgett film, functionalized glass, germanium, silicon, polymers, (poly)tetrafluoroethylene, polystyrene, gallium arsenide, and combinations thereof.

9. The method as recited in claim 1 wherein said protective group is selected from the group consisting of ortho-nitrobenzyl derivatives, 6-nitroveratryloxy-carbonyl, 2-nitrobenzyloxycarbonyl, cinnamoyl derivatives, and mixtures thereof.

10. The method as recited in claim 1 wherein said first and second regions each have total areas of less than 1 cm^2 .

11. The method as recited in claim 1 wherein said first and second regions each have total areas of between about $1 \text{ }\mu\text{m}^2$ and $10,000 \text{ }\mu\text{m}^2$.

12. The method as recited in claim 4 wherein said light is monochromatic coherent light.

13. The method as recited in claim 1 wherein said steps of exposing to an activator are carried out with a solution in contact with said substrate.

14. The method as recited in claim 13 wherein said solution further comprises said first or said second monomer.

15. The method as recited in claim 6 wherein said receptor further comprises a marker selected from the group consisting of radioactive markers and fluorescent

markers and wherein said step of testing for the presence of the receptor is a step of detecting said marker.

5 16. The method as recited in claim 1 wherein the steps of exposing to an activator further comprise steps of:

a) placing a mask adjacent to said substrate, said mask having substantially transparent regions and substantially opaque regions at a wavelength of light; and

10 b) illuminating said mask with a light source, said light source producing at least said wavelength of light.

15 17. The method as recited in claim 1 wherein said steps are repeated so as to synthesize 10^3 or more different sequences on said substrate.

20 18. The method as recited in claim 1 wherein said steps are repeated so as to synthesize 10^6 or more different sequences on said substrate.

25 19. A method of synthesizing a plurality of chemical sequences, said chemical sequences comprising at least a first and a second monomer, comprising the steps of:

30 a) at a first region on a substrate having at least a first and a second region, said first and said second region comprising a substrate protective group, activating said first region to remove said substrate protective group in said first region;

35 b) exposing said first monomer to said substrate, said first monomer further comprising a first monomer protective group, said first monomer binding at said first region;

c) activating said second region to remove said substrate protective group in said second region;

d) exposing said second monomer to said substrate, said second monomer further comprising a second monomer protective group, said second monomer binding at said second region;

5 e) activating said first region to remove said first monomer protective group;

f) exposing a third monomer to said substrate, said third monomer binding at said first region to produce a first sequence;

10 g) activating said second region to remove said second monomer protective group; and

h) exposing a fourth monomer to said substrate, said fourth monomer binding at said second region to produce a second sequence, said second sequence
15 different from said first sequence.

20 20. A method of synthesizing a plurality of chemical sequences, said chemical sequences comprising at least a first and a second monomer, comprising the steps of:

a) on a substrate having at least a first and a second region deactivating said first region to provide a first protective group in said first region;

25 b) exposing said first monomer to said substrate, said first monomer binding at said second region;

c) removing said protective group in said first region;

30 d) deactivating said second region to provide a second protective group in said second region;

e) exposing said second monomer to said substrate, said second monomer binding at said first region;

35 f) removing said protective group in said second region;

g) deactivating said first region to provide a protective group in said first region;

h) exposing a third monomer to said substrate, said third monomer binding at said second region to produce a first sequence;

i) removing said protective group in said first region; and

j) exposing a fourth monomer to said substrate, said fourth monomer binding at said first region to produce a second sequence, said second sequence different than said first sequence.

21. A method of synthesizing at least a first polymer sequence and a second polymer sequence on a substrate, said first polymer sequence having a different monomer sequence from said second polymer sequence, comprising the steps of:

a) inserting a first mask between said substrate and an energy source, said mask having first regions and second regions, said first regions permitting passage of energy from said source, said second regions blocking energy from said source;

b) directing energy from said source at said substrate, said energy removing a protective group from first portions of said first polymer under said first regions of said first mask;

c) exposing a second portion of said first polymer to said substrate to create a first polymer sequence;

d) inserting a second mask between said substrate and said energy source, said second mask having first regions and second regions;

e) directing energy from said source at said substrate, said energy removing said protective group under said first regions of said second mask from first portions of said second polymer; and

f) exposing a second portion of said second polymer to said substrate, said second portion of said

second polymer binding with said first portion of said second polymer to create a polymer 8second sequence.

5 22. The method as recited in claim 21 wherein said energy is selected from the group consisting of ion beams, electron beams, gamma rays, x-rays, ultra-violet radiation, light, infra-red radiation, microwaves, electric fields, radiowaves, and combinations thereof.

10 23. The method as recited in claim 19 wherein said protective groups are photosensitive protective groups.

15 24. The method as recited in claims 19 or 20 wherein said steps of activating and deactivating are steps of applying light to selected regions of said substrate.

20 25. The method as recited in claims 19 or 20 wherein said first and said second monomers are amino acids.

25 26. The method as recited in claims 19, 20 or 21 further comprising a step of screening said first and said second sequences for affinity with a first receptor, said step of screening further comprising a step of exposing said substrate to said first receptor and testing for the presence of said first receptor.

30 27. The method as recited in claim 26 wherein said step of screening is a step of screening with antibodies.

35 28. The method as recited in claims 19, 20 or 21 wherein said substrate is selected from the group consisting of a polymerized Langmuir Blodgett film, functionalized glass, germanium, silicon, polymers, (poly)tetrafluoroethylene, gallium arsenide, gallium

phosphide, silicon oxide, silicon nitride and combinations thereof.

29. The method as recited in claim 19 wherein said protective group, said first monomer protective group, and said second monomer protective group are selected from the group consisting of ortho-nitrobenzyl derivatives, 6-nitroveratryloxycarbonyl, 2-nitrobenzyloxycarbonyl, and mixtures thereof.

30. The method as recited in claim 20 wherein said protective group is a cinnamate group.

31. The method as recited in claims 19 or 20 wherein said first and second regions each have total areas of less than 1 cm².

32. The method as recited in claims 19 or 20 wherein said first and second regions each have total areas of between about 1 μm² and 10,000 μm².

33. The method as recited in claim 24 wherein said light is monochromatic coherent light.

34. The method as recited in claim 19 wherein said steps of activating are carried out with a solution in contact with said substrate.

35. The method as recited in claim 34 wherein said solution further comprises a monomer.

36. The method as recited in claim 26 wherein said receptor further comprises a marker selected from the group consisting of radioactive markers and fluorescent markers and wherein said step of testing for the presence of the receptor is a step of detecting said marker.

37. The method as recited in claims 19 or 20 wherein two of said first, said second, said third, and said fourth monomers are the same monomers.

5 38. The method as recited in claim 21 wherein the step of inserting a second mask is a step of translating said first mask from a first position to a second position.

10 39. The method as recited in claim 21 wherein the step of inserting a second mask is a step of rotating said first mask.

15 40. The method as recited in claim 26 further comprising the step of exposing said substrate to a second, labeled receptor, said second, labeled receptor binding at multiple sites on said first receptor.

20 41. The method as recited in claim 40 wherein said first receptor is an antibody of a first animal species and said second receptor is an antibody derived from a second species and directed at said first species.

25 42. The method as recited in claim 19 wherein:

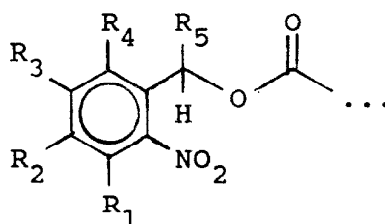
a) said first monomer protective group is removable upon exposure to a first wavelength of light;

b) said second monomer protective group is removable upon exposure to a second wavelength of light;

30 c) said step of activating said first region to remove said first monomer protective group is a step of exposing substantially all of said substrate to said first wavelength of light; and

35 d) said step of activating said second region to remove said second monomer protective group is a step of exposing substantially all of said substrate to said second wavelength of light.

43. A method as recited in claims 19 or 21 wherein said protective groups are of the form:



where R₁ is alkoxy, alkyl, halo, aryl, alkenyl, or hydrogen; R₂ is alkoxy, alkyl, halo, aryl, nitro, or hydrogen; R₃ is alkoxy, alkyl, halo, nitro, aryl, or hydrogen; R₄ is alkoxy, alkyl, hydrogen, aryl, halo, or nitro; and R₅ is alkyl, alkynyl, cyano, alkoxy, hydrogen, halo, aryl, or alkenyl.

44. A method of screening a plurality of amino acid sequences for binding with a receptor comprising the steps of:

- a) on a glass plate having at least a first surface, said at least a first surface comprising a photoprotective material selected from the group consisting of nitroveratryloxy carbonyl and nitrobenzyloxy carbonyl, reacting said at least a first surface with t-butoxycarbonyl for storage, said glass plate substantially transparent to at least ultraviolet light;
- b) exposing said at least a first surface to TFA to remove said t-butoxycarbonyl;
- c) placing said glass plate on a reactor, said reactor comprising a reactor space, said at least a first surface exposed to said reactor space;
- d) placing a mask at a first position on said glass plate, said mask comprising first locations and second locations, said first locations substantially transparent to at least ultraviolet light and said second locations substantially opaque to at least ultraviolet

light, said second locations comprising a light blocking material on a first surface of said mask, said first surface of said mask placed in contact with said glass plate;

5 e) filling said reactor space with a reaction solution;

f) illuminating said mask with at least ultraviolet light, said ultraviolet light removing said photoprotective material from said at least a first surface of said glass plate under said first locations of said mask;

g) exposing said first surface to a first amino acid, said first amino acid binding to regions of said at least a first surface from which said photoprotective material was removed, said first amino acid comprising said photoprotective group at a terminus thereof;

h) placing a mask in contact with said glass plate at a second position;

20 i) illuminating said mask with at least ultraviolet light, said ultraviolet light removing said photoprotective material from said at least a first surface of said glass plate under said first locations of said mask;

25 j) exposing said at least a first surface to a second amino acid, said second amino acid binding to regions of said at least a first surface from which said photoprotective material was removed, said second amino acid comprising said photoprotective group at a terminus thereof;

30 k) placing a mask in contact with said glass plate at a third position;

l) illuminating said mask with at least ultraviolet light, said ultraviolet light removing said photoprotective material from said at least a first surface of said glass plate under said first locations of said mask;

m) exposing said at least a first surface to a third amino acid, said third amino acid binding to regions of said at least a first surface from which said photoprotective material was removed;

5 n) placing a mask in contact with said glass plate at a fourth position;

o) illuminating said mask with at least ultraviolet light, said ultraviolet light removing said photoprotective material from said at least a first surface of said glass plate. Under said first locations of said mask;

10 p) exposing said at least a first surface to a fourth amino acid, said fourth amino acid binding to regions of said at least a first surface from which said photoprotective material was removed, said at least a first surface comprising at least first, second, third, and fourth amino acid sequences;

15 q) exposing said at least a first surface to an antibody of interest, said antibody of interest binding more strongly to at least one of said first, said second, said third, or said fourth amino acid sequences;

20 r) exposing said at least a first surface to a receptor, said receptor recognizing said antibody of interest and binding at multiple locations thereof, said receptor comprising fluorescein;

25 s) exposing said at least a first surface to light, said first surface fluorescing in at least a region where said more strongly bound amino acid sequence is located; and

30 t) detecting and recording fluoresced light intensity as a function of location across said at least a first surface.

45. A method of identifying at least one peptide sequence for binding with a receptor comprising the steps of:

5 a) on a substrate having a plurality of polypeptides, each having a photoremovable protective group, irradiating first selected polypeptides to remove said protective group;

10 b) contacting said polypeptides with a first amino acid to create a first sequence, second polypeptides on said substrate comprising a second sequence; and

c) identifying which of said first or said second sequence binds with said receptor.

15 46. The method as recited in claim 45 wherein said step of identifying further comprises a step of detecting the presence of a marker selected from the group consisting of radioactive markers and fluorescent markers in said receptor.

20 47. The method as recited in claim 45 wherein said step of irradiating is a step of masking a light source with a mask, said mask comprising first transparent regions and second opaque regions.

25 48. The method as recited in claim 47 wherein the step of identifying further comprises the steps of:

a) exposing a first receptor to said substrate; and

30 b) exposing a receptor to said first receptor to said substrate, said receptor to said first receptor comprising a marker.

35 49. The method as recited in claim 48 wherein said marker is selected from the group consisting of radioactive markers and fluorescent markers.

50. The method as recited in claim 48 wherein said first receptor is an antibody from a first species and said receptor to said first receptor is an antibody from a second species directed at said first species.

5 51. A method for screening a plurality of polymers for biological activity comprising exposing a receptor to a substrate having said plurality of said polymers on a surface thereof, each of said polymers occupying an area
10 of less than about 1 cm².

52. A method for screening as recited in claim 48 wherein said area is less than about 0.1 cm².

15 53. A method as recited in claim 48 wherein said area is less than about 10,000 μm².

54. A method as recited in claim 48 wherein said area is less than about 100 μm².

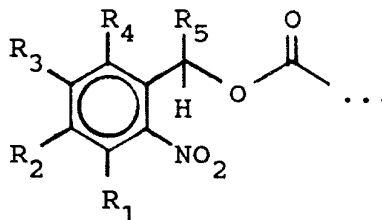
20 55. Apparatus for preparation of a plurality of polymers comprising:

a) a substrate with a surface, said surface comprising a reactive portion, said reactive portion
25 activated upon exposure to an energy source so as to react with a monomer; and

b) means for selectively protecting and exposing portions of said surface from said energy source.

30 56. Apparatus as recited in claim 55 wherein said reactive portion further comprises a protective group, said protective group of the form:

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where R₁ is alkoxy, alkyl, halo, aryl, alkenyl, or hydrogen; R₂ is alkoxy, alkyl, halo, aryl, nitro, or hydrogen; R₃ is alkoxy, alkyl, halo, nitro, aryl, or hydrogen; R₄ is alkoxy, alkyl, hydrogen, aryl, halo, or nitro; and R₅ is alkyl, alkynyl, cyano, alkoxy, hydrogen, halo, aryl, or alkenyl.

57. Apparatus as recited in claim 55 wherein said reactive portion further comprises linker molecules.

58. Apparatus as recited in claim 57 wherein said linker molecules are selected from the group consisting of ethylene glycol oligomers, diamines, diacids, amino acids, and combinations thereof.

59. Apparatus as recited in claim 55 wherein said means for selectively protecting further comprises a mask.

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60. Apparatus as recited in claim 55 wherein said means for selectively protecting further comprises a light valve.

61. Apparatus as recited in claim 55 wherein said energy source is a light source.

62. Apparatus as recited in claim 55 wherein said reactive portion further comprises a composition selected from the group consisting of nitroveratryloxy carbonyl, nitrobenzyloxy carbonyl, dimethyl-dimethoxybenzyloxy

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carbonyl, 5-bromo-7-nitroindolyl, hydroxy-2-methyl cinnamoyl, and 2-oxymethylene anthraquinone.

63. Apparatus for preparation of a substrate having a plurality of amino acid sequences thereon, said apparatus comprising:

- a) a substrate with a surface;
- b) a protective group on said surface, said protective group removable upon exposure to an energy source, said energy source selected from the group consisting of light, electron beams, and x-ray radiation;
- c) means for directing said energy source at selected locations on said surface; and
- d) means for exposing amino acids to said surface for binding to said surface.

64. Apparatus for screening polymers comprising a substrate with a surface, said surface comprising at least two predefined regions, said predefined regions containing different monomer sequences thereon, said predefined regions each occupying an area of less than about 0.1 cm^2 .

65. Apparatus as recited in claim 64 wherein said area is less than about 0.01 cm^2 .

66. Apparatus as recited in claim 64 wherein said area is less than $10000 \text{ } \mu\text{m}^2$.

67. Apparatus as recited in claim 64 wherein said area is less than about $100 \text{ } \mu\text{m}^2$.

68. Apparatus as recited in claims 64, 65, 66, or 67 wherein said monomer sequences are substantially pure within said predefined regions.

69. A substrate for screening for biological activity, said substrate comprising 10^3 or more different ligands on a surface thereof in predefined regions.

5 70. A substrate as recited in claim 69 wherein said substrate comprises 10^4 or more different ligands in predefined regions.

10 71. A substrate as recited in claim 69 wherein said substrate comprises 10^5 or more different ligands in predefined regions.

15 72. A substrate as recited in claim 69 wherein said substrate comprises 10^6 or more different ligands in predefined regions.

 73. A substrate as recited in claims 69, 70, 71, or 72 wherein the ligands are peptides.

20 74. A substrate as recited in claim 64 wherein said ligands are substantially pure within said predefined regions.

25 75. Apparatus for screening for biological activity comprising:

30 a) a substrate comprising a plurality of polymer sequences, said polymer sequences attached to a surface of said substrate at known locations on said substrate, each of said sequences occupying an area of less than about 0.1 cm^2 ;

 b) means for exposing said substrate to a receptor, said receptor marked with a fluorescent marker, said receptor binding with at least one of said sequences; and

35 c) means for detecting a location of said fluorescent marker on said substrate.

76. Apparatus for forming a plurality of polymer sequences comprising:

5 a) a substrate, said substrate having at least a first surface and a second surface, said second surface comprising a photoremovable protective material, said substrate substantially transparent to at least light of a first wavelength;

10 b) a reactor body, said reactor body having a mounting surface with a reaction fluid cavity therein, said second surface maintained in a sealed relationship with said mounting surface; and

15 c) a light source for producing light of at least said first wavelength and directed at a surface of said substrate.

77. Apparatus as recited in claim 76 wherein said light source is directed at said first surface.

20 78. Apparatus as recited in claim 76 further comprising a mask, said mask placed between said light source and said first surface, said mask having first regions substantially transparent to said first wavelength of light and second regions substantially opaque to said first wavelength of light.

25 79. Apparatus as recited in claim 76 wherein said cavity comprises a fluid inlet and a fluid outlet, said fluid inlet connected to a pump for flowing reaction fluids through said cavity.

30 80. Apparatus as recited in claim 76 wherein said cavity further comprises a plurality of raised sections.

35 81. Apparatus as recited in claim 78 wherein said mask further comprises a glass plate.

82. Apparatus as recited in claim 81 wherein said opaque regions on said mask comprise chrome.

5 83. Apparatus as recited in claim 76 wherein at least a portion of said second surface comprises a second photoremovable protective group, said second photoremovable protective group activatable upon exposure to light of a second wavelength.

10 84. Apparatus as recited in claim 76 further comprising first and second gaskets on said mounting surface and means for maintaining a vacuum between said first and second gaskets.

15 85. Apparatus as recited in claim 76 wherein said substrate has a thickness of less than 1 mm.

20 86. Apparatus as recited in claim 76 wherein said substrate has a thickness of less than 0.5 mm.

87. Apparatus as recited in claim 76 wherein said substrate has a thickness of less than 0.05 mm.

25 88. Apparatus as recited in claim 78 wherein said mask is in direct contact with said substrate.

89. Apparatus as recited in claim 88 wherein opaque regions of said mask are placed in direct contact with said substrate.

30 90. Apparatus as recited in claim 76 further comprising a liquid crystal light valve for selectively controlling exposure of light to said substrate.

35 91. Apparatus as recited in claim 76 further comprising a fiber optic faceplate between said light source and said substrate.

92. Apparatus as recited in claim 76 further comprising a molecular microcrystal between said light source and said substrate.

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93. Apparatus as recited in claim 76 wherein said cavity comprises light absorptive materials.

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94. Apparatus as recited in claim 93 wherein said light absorptive material is N,N-diethylamino 2,4-dinitrobenzene.

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95. Apparatus as recited in claim 76 wherein said cavity is filled with a carrier solution.

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96. Apparatus as recited in claim 95 wherein said carrier material comprises a material selected from the group of 1-hydroxybenzotriazole, dimethylformamide, diisopropylethylamine, and benzotriazolyl-n-oxy-tris(dimethylamino)phosphoniumhexafluorophosphate.

97. Apparatus as recited in claim 76 wherein said substrate is a fiber optic faceplate.

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98. Apparatus for detection of fluorescent marked regions on a substrate comprising:

a) a light source for directing light at a surface of said substrate;

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b) a means for detecting light fluoresced from said surface in response to said light source;

c) means for translating said substrate from a first position to a second position; and

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d) means for storing fluoresced light intensity as a function of location on said substrate, said means for storing connected to said means for translating and said means for detecting.

99. Apparatus as recited in claim 98 further comprising video display means for displaying light intensity as a function of location on said substrate.

5 100. Apparatus as recited in claim 98 wherein said means for detecting comprises a photomultiplier tube and a photon counter.

10 101. Apparatus as recited in claim 99 wherein said means for directing light further comprises a dichroic mirror, said mirror reflecting light at a wavelength of said light source and passing said fluoresced light.

15 102. Apparatus as recited in claim 100 wherein said light source is a laser light source.

 103. Apparatus as recited in claim 101 wherein said means for storing is a programmed digital computer.

20 104. Apparatus as recited in claim 102 further comprising a microscope, said light source directed at said substrate through said microscope, said means for detecting receiving light from said microscope.

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VERY LARGE SCALE IMMOBILIZED POLYMER SYNTHESIS

ABSTRACT OF THE DISCLOSURE

5 A method and apparatus for preparation
of a substrate containing a plurality of sequences.
Photoremovable groups are attached to a surface of a
substrate. Selected regions of the substrate are exposed
10 to light so as to activate the selected areas. A
monomer, also containing a photoremovable group, is
provided to the substrate to bind at the selected areas.
The process is repeated using a variety of monomers such
as amino acids until sequences of a desired length are
15 obtained. Detection methods and apparatus are also
disclosed.

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